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IN *VITRO* ANTI-DIABETIC PROPERTIES FROM DIFFERENT PLANT SOURCES

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ABSTRACT:
α-amylase is an enzyme that plays a vital role in the carbohydrate digestion. One antidiabetic therapeutic approach reduces the post prandial glucose level in blood by the inhibition of alpha-amylase enzymes. These can be an important strategy in management of blood glucose. The aim of the present study is to investigate the phytochemical bioactive compounds of the ethanoic extract of various plant extract, its in vitro anti-diabetic activity. The assay result suggests that the presence of bioactive compounds could be responsible for the versatile medicinal purpose. Properties of these plants includes anti-diabetic effect, the extract exhibit the dose-dependent increase in inhibitory effect on alpha-amylase enzyme.

KEYWORDS:-*Alpha-amylase, in vitro anti-diabetic properties, Plant sources, Leaf extract, Enzyme Activity.*

INTRODUCTION

Diabetes Mellitus aheterogeneous metabolic disorder is characterized by hyperglycemia due to defective insulin secretion, resistance to insulin action or both. Management of diabetes without any side effect is still a challenge to the medical community. Here we are using some medicinal plants which provide the useful source of hypoglycemic compounds for the development of pharmaceutical entities or as an adjunct to existing therapies***. Tritium*** (Wheat grass), ***Hibiscus waimeae*** (white china rose),***Calotropis gigantean***(Crown flower stem) are such medicinal plant which are being explored for their hypoglycemic property. Plants are being continuously explored for their possible effect as hypoglycaemic agents (Bailey and Day., 1989). Antidiabetic effect of plants and their active principles can be assessed in vitro using a variety of biological test systems. They play a major role in evaluation of antidiabetic properties as an initial screening tool prior to in *vivo* studies. The present review focuses on in *vitro* assays that are available to study potential antidiabetic activity of plant extracts and their active constituents. It is evident that these plant extract and compounds derived from them are capable of lowering blood glucose level through different mechanism of action. This has attracted a great deal of research interest in exploring natural sources. In *vitro* assay provides a basic platform for accusing these plant extracts and help us understand various mechanisms that would alleviate hyperglycaemia in diabetes.In *vitro* models are fairly based on a specific process, wherein activity of an enzyme on a metabolic reaction or binding to a receptor within a given cell type can be studied. In *vitro* studies are of considerable value in identifying the mechanism of action of a test material and are more economical. It provides an alternative to animal testing in many aspects which includes investigation of organs or tissues derived from animals that could be used to test many replicates or samples.

MATERIAL AND METHODS

Collection and authentication of samples

Fresh leaves of the hibiscus, fresh stem of crown flower were collected from the Bichpuri village, Uttar Pradesh, India and the wheat grass had been grown at Raja Balwantsingh engineering technical campus BichpuriAgra,Uttar Pradesh India, and the seeds for the same were collected from the Singhal Provisional store , AyodhyaKunj, U.P,India.

Preparation of sample for extraction

The authenticated plant samples were washed and driedby using vacuum oven at 40^{o} C until all the water and moister is vaporized and ground into powder. It took 48 hours for Hibiscus leaves, 40 hours for Crown flower stem and 52 hours for Wheat grass drying. Extract was prepared by successive maceration of the powder (2 gm) at room temperature with ethanol extract (20 ml) in the shaker for 2 days (Arunachalam et al., 2013). The extract obtained was Centrifuge at 4^{o}C for 15 minute at 10,000 RPM .The final extract obtained was filtered and the filtrate was kept in refrigerator at 4^{o}C (Anju And Nidhi., 2016).

Testing of sample for α-amylase activity inhibition

Three concentration of each different sample were prepared. These were 0.5ml, 1ml, and 2ml.Then 20µl of α-amylase was added and incubates for 10 minute at 37^{o}C. After pre-incubation, 200µl of 1% starch solution was added to each test tube and incubated for 1 hour at 37^{o}C. A volume of 200µl of 1% iodine solution with 10ml of distilled water was added to each test tube. Finally this reaction mixture was used to measure absorbance at 565nm.

Percentage inhibition = $\frac{[A(control\ ) - A(extraction\ )]}{A(control\ )} * 100$
A = absorbance **Control = 0.46**

TABLE 1: Percentage inhibition of α-amylase at varying concentration of Hibiscus leaves

S.NO	CONCENTRATION	OPTICAL DENSITY	PERCENTAGE INHIBITION
1	2ML	0.30	34.78%
2	1ML	0.29	36.95%
3	0.5ML	0.40	13.04%

TABLE 2: Percentage inhibition of α-amylase at varying concentration of crown flower stem

S.NO	CONCENTRATION	OPTICAL DENSITY	PERCENTAGE INHIBITION
1	2ML	0.31	58.69%
2	1ML	0.30	45.65%
3	0.5ML	0.32	13.04%

TABLE 3: Percentage inhibition of α-amylase at varying concentration of wheat grass

S.NO	CONCENTRATION	OPTICAL DENSITY	PERCENTAGE INHIBITION
1	2ML	0.2	56.52%
2	1ML	0.19	58.69%
3	0.5ML	0.34	26.08%

RESULT

At the Concentration of 0.5ml/ml (0.5 ml of plant extract in 1 ml of distilled water) wheat grass showed maximum percentage inhibition of the enzyme with the highest value of 26.08%(Table 3).While at the concentration of 1ml/ml (1 ml extract in 1 ml of distilled water) wheat grass again showed maximum percentage inhibition of the enzyme with highest value of 58.69%(Table 4), but at the concentration of 2ml/ml (2ml of extract in 1 ml of distilled water) crown flower stem showed the maximum percentage inhibition of the enzyme with highest value of 58.69%.

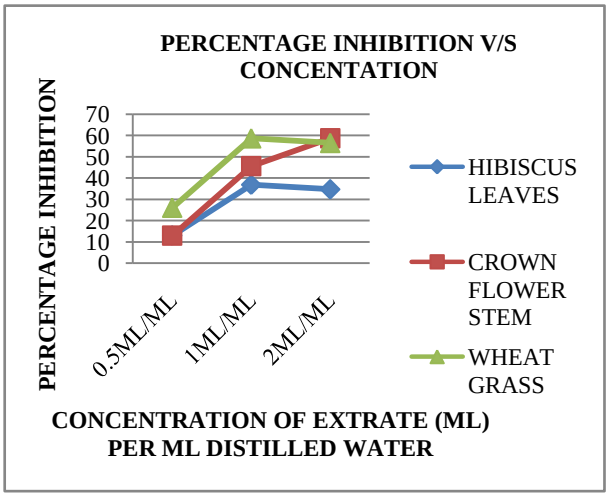

Fig.1 Effect of different concentration of ethanol extract of Hibiscus leaves, Crown flower stem and Wheat grass on in *vitro* α- amylase inhibition test. The figure is in percentage showing the effect on in *vitro* α-amylase activity

CONCLUSION

I*n vitro* condition of extracts showed excellent inhibition of the α-amylase activity. Determination of the natural antidiabetic compound of the plant extract will help to develop new drug candidates for antidiabetic therapy. The plants would consider as good sources of natural antidiabetants for medicinal uses. Natural products such as plant extracts, phytochemicals, and microbial metabolites are attracting more and more attention for their potential uses in the treatment and prevention of diabetes. A number of plant extracts and natural biomolecules that have been tested for their antidiabetic properties using in *vitro* approaches were reviewed here. Some of them show very promising effects, which indicate that the dietary intake of phytochemicals could be a promising strategy for diabetes prevention.

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